UNIVERSITY OF ALBERTA

FORMATION-PLUGGING MICROORGANISMS IN OIL FIELD INJECTION WATERS

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ABSTRACT

Injection waters of Pembina and other Alberta water flood projects were investigated to determine the major types and numbers of microorganisms present. Samples for analysis were obtained from different points throughout the various injection systems with the majority of samples coming from injection well heads.

The methods used in the investigation and their specificity are discussed. Particular attention is paid to the detection of iron-depositing bacteria and sulfate-reducing bacteria. The results indicate that fungi, algae, diatoms, actinomycetes, and protozoa are of little concern in causing the accumulation of organic matter. Sulfate-reducing bacteria, iron-depositing bacteria and high counts of viable bacteria including slime formers, have been frequently detected at various points in different injection systems. From the bacteriological point of view, well and surface waters do not seem to differ in suitability for injection purposes.

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INTRODUCTION

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Several methods are employed to recover the oil remaining in petroliferous formations after the yield by conventional means has dropped below that which is profitable for commercial production. One of the most frequently employed methods is the flooding operation in which water is used to displace oil. The possibility of using such a technique was discovered accidentally some sixty years ago when it was noticed that leakage of water into one well could result in an increased oil production in surrounding wells (Power, 1951). Subsequently, fresh water injection became one of the main operations used in the production of oil. Brine injection was not practiced until much later. However, one serious complication occurred with the use of this technique: the rate of injection of the flood waters could decrease and the pressure at the well head increase. This suggested that a plugging of the formation was taking place. A number of factors may contribute to such plugging but the most important are the chemical and microbiological quality of the injection water. Such plugging may call for costly and time consuming cleaning of the injection well, a procedure, which, if not successful , may result in loss of recoverable oil.

The work reported here consists of a survey of the microbiological flora of the waters used for flooding operations in Alberta oil fields and of an evaluation of the techniques employed for the microbiological testing of these waters.

HISTORICAL REVIEW

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1. General.

Fresh water derived from surface wells or nearby streams and reservoirs has been principally used in flooding operations. Water which was clear at the source was often found to be cloudy by the time it reached the injection well and back flowed water from the injection well head was frequently opaque (Beck, 1946). The continued use of such water often led to a reduction of the injection rate and an increased pressure at the well head indicating that plugging of the formation was occurring. Since the appearance of suspended matter between the water source and the injection well was attributed to "chemical reactions", methods to control these reactions by treatment of the water were developed. However, these treatments could only reduce the rate of injection decline but could not prevent it entirely. Subsequently, it was frequently noticed that much of the suspended matter was of organic nature. Closer investigation revealed that the organic matter consisted of cellular material such as the common micro-organisms of the air (e.g., Bacillus subtilis), as well as sulfate-reducing bacteria, sulfur bacteria, iron bacteria, fungi, algae and diatoms. Inorganic precipitates such as iron sulfide, iron hydroxide, and calcium carbonate have also been reported. These are insoluble metabolic products of some of the organisms listed above (Beerstecher, 1954).

The majority of the suspended material in the injection water will be filtered out at the sand face of the fine petroliferous formation. Some finer particulate matter, including microorganisms and their products, will selectively plug smaller pores within the formation (Merkt, 1943; Beck, 1947; Fekette, 1959). Correlation between bacterial numbers and the rate of plugging of an injection well was demonstrated by Beck (1947). He reported that the use of injection water which had a bacterial count of 20,000 to 50,000 organisms per ml caused the injection rate to decrease to three to four barrels per day. A comparable well in which the injection water had a bacterial count of 10 to 1,000 organisms per ml could, after the same lapse of time, take 20 barrels per day.

2. Principal Organisms Causing Formation Plugging.

The plugging of formations by injection water can be attributed primarily to the presence in the water of three groups of organisms: sulfate-reducing bacteria, iron bacteria, and slime-producing bacteria. A description of these organisms follows.

a) Sulfate-reducing bacteria.

i) Description

The majority of sulfate-reducing bacteria belong to the genus Desulfovibrio. The characteristic property of bacteria belonging to this genus is their ability to utilize sulfate as their final hydrogen or electron acceptor thus producing hydrogen sulfide. They are Gram negative, slightly curved, non-sporing, actively motile bacilli with a single terminal flagellum. They are from 0.5 to 1 micron wide and 1 to 5 microns long. Organisms occur singly or sometimes in short chains having a spiral appearance. The prototype of this group, D. desulfuricans, was first isolated and named by Beijerinck in 1895 and since then they have intrigued many workers. At present there are several species and strains known which constitute the genus Desulfovibrio.

The sulfate-reducing bacteria, as a group, tolerate a wide range of salt concentrations. They are found in fresh water, sea water, and brine solutions obtained with oil at the producing well. ZoBell (1958) found that sulfate-reducing bacteria isolated from soil, sewage, and fresh water are active in physiological saline solution but are inhibited by salt concentrations of 1.5% and up. Optimum

growth of organisms isolated from sea water occurs in 3% salt solution. Some strains which ZoBell has isolated tolerate up to 30% sodium chloride. Salt tolerance, however, is affected by temperature, pressure, hydrogen ion concentration, and other factors.

The sulfate reducers grow and multiply in the temperature range of -10° to 100°C. Bacteria growing naturally at -3° to -11°C have been reported by ZoBell (1934). However, these organisms were observed to grow better at 20° to 30°C rather then at the low temperature from which they were isolated. Butlin, Adams, and Thomas (1949a) reported that media inoculated with a soil sample showed growth at 30°, 35°, 40°, 45°, 50°, and 55°C, while a pure culture would not grow at all at these temperatures without acclimatization. ZoBell (1958) observed that some sulfate-reducing bacteria will grow at temperatures between 65° and 85°C when under hydrostatic pressures of 200 to 400 atmospheres. Other cultures were found which could grow at 1,000 atmospheres and 104°C. There are, however, no true thermophilic strains in the genus Desulfovibrio.

Sulfate reducing bacteria in their natural habitat are subjected to various hydrostatic pressures. ZoBell (1958) reported that organisms isolated from sewage and soil were inhibited by a pressure of 400 to 600 atmospheres when kept at their optimum growth temperature. Sulfate-reducing bacteria isolated from sea sediment, however, failed to multiply unless they were incubated under hydrostatic pressures of 700 or 1,000 atmospheres. Strains isolated from oil well

cores withstood pressures up to 600 atmospheres and temperatures up to 85°C, even though they were neither barophilic nor thermophilic. An interesting phenomenon was observed by ZoBell concerning a certain strain of Desulfovibrio which showed increased hydrogen sulfide production with an increase of pressure up to 1,000 atmospheres.

Optimum growth of sulfate reducers occurs at neutral reaction. They have been found, however, to be active in culture media ranging in pH from 5.5 to 8.5 (Starkey and Wight, 1945). Anderson, Lanigan, Liegey, Worden, Yackovich, and Finan (1958) reported that complete control of these organisms in flood operation can only be achieved with a pH of 2.5 to 3.5.

One of the most significant factors affecting growth of Desulfovibrio is the redox potential. In situ, where sulfate reducers were found actively growing, the Eh value was invariably between -100 and -300 millivolts (ZoBell, 1958). It is suspected that the reason no growth has occasionally been reported in media inoculated with Desulfovibrio was due to the fact that the required redox potential was not attained. A medium giving the desired Eh value by the use of a combination of ascorbic acid and sodium thioglycolate has been described by Allred, Mills, and Fisher (1954).

Desulfovibrio utilize various organic substances as their hydrogen donors. Starkey (1958) lists such materials as simple carbohydrates, organic acids, alcohols, and amino acids. It had been found that different cultures utilize

different substrates and this fact has been used in the differentiation of the species. Novelli and ZoBell (1944) have shown that sulfate reducers will utilize petroleum hydrocarbons, only those larger than decane but will not attack aromatic groups. Utilization of molecular hydrogen has been reported by Butlin and Adams (1947), Sisler and ZoBell (1950), and Postgate (1951). Reduction of sulfate to sulfide under heterotrophic and autotrophic conditions has been represented by the following equations (ZoBell, 1958):

$$2C_3H_5O_3Na + MgSO_4 \longrightarrow H_2S + 2C_2H_3O_2Na + MgCO_3 + CO_2 + H_2O$$

$$4H_2 + MgSO_4 \longrightarrow H_2S + MgO + 3H_2O$$

Nitrogen requirements are met by ammonium compounds which can not be replaced by nitrates or nitrites (ZoBell, 1958). Sisler and ZoBell (1951) reported that molecular nitrogen can also be utilized.

Sulfate reducers are tolerant of high concentrations of hydrogen sulfide. In the Black Sea they produce such high quantities of H_2S that all other forms of life are inhibited (ZoBell, 1958). Sulfate-reducing bacteria have also been isolated from produced water of high sulfide content (Ginter, 1934). Experimentally it has been shown that adding organic matter, such as lactate, to the medium increased production of hydrogen sulfide (Miller, 1950).

According to the growth requirements listed in the preceding paragraphs it is not surprising to find sulfate-reducing bacteria active in petroliferous formations all over the world. This may not be a natural habitat but may be due to their introduction by man (ZoBell, 1958). In

California and Illinois, Bastin (1926) recovered sulfate reducers from 43 different oil wells. Young (1936) isolated cultures of sulfate-reducing bacteria from water of shallow oil wells in Alberta. Issatchenko, Reinfeld and Sturm (1945), detected sulfate reducers in produced waters from Baku and Kirigiziya oil fields. Out of 396 samples obtained in California, Texas, Louisiana, Oklahoma, Illinois, and Pennsylvania, ZoBell (1958) found 187 positive for Desulfovibrio. Samples collected by ZoBell came from production zones ranging from 2,680 to 10,470 feet below the earth's surface.

The sulfate-reducing bacteria are susceptible to certain antibiotics. A broad spectrum antibiotic (terramycin) was tested in a flooding operation reported by Anderson et al. (1958). He found the treatment beneficial and suggested that it could be used where strains of bacteria have developed which are resistant to certain bactericides.

ii) Corrosive activities.

Anaerobic corrosion, as defined by van Wolzogen Kuhr and van der Vlugt (1934), is that rapid deterioration of iron metal under anaerobic conditions which would not occur or would be insignificant in the absence of Desulfovibrio. The theory proposed by these two workers to explain the reaction is as follows: Iron metal will tend to go into solution until an equilibrium is reached between the iron and hydrogen ions. If this state is maintained there will be no further loss of the metal. Sulfate-reducing bacteria, present on the metal surface, will utilize cathodic hydrogen

causing more iron to go into solution resulting in corrosion. Products of this reaction were found to be iron sulfide and iron hydroxide. An overall equation explaining this reaction is given as:

$$4Fe + Na_2SO_4 + 4H_2O \longrightarrow FeS + 3Fe(OH)_2 + 2NaOH$$

There are factors supporting the above theory and others in conflict with it as pointed out by Starkey (1958).

Anaerobic corrosion has been observed to occur in soils, fresh and salt water and on iron equipment such as pipe lines, pumps, well casings and others. Internal corrosion of iron pipes conveying fresh water has been discussed by Butlin, Adams and Thomas (1949b). Blanton and Oppenheimer (1960) reported a 66% increase in corrosion of iron in cultures of Desulfovibrio over uninoculated control tubes. In one experimental field trial, pipe-line corrosion was reduced by 40 to 80% within two years by the use of bactericides which had no corrosion inhibiting property (Allred, 1957).

iii) Plugging effect.

The presence of sulfate-reducing bacteria in injection systems of the Bradford area was manifested by the presence of "rotten-egg gas" and "black water". Using such water in flooding operations often resulted in a rapid decline of the injection rates (Beck, 1947). An experiment carried out by Beck in flooding a tight core for one day with a culture of sulfate reducers brought about 25% reduction in intake rate. Merkt (1943) flooded walnut oolithic limestone cores with untreated produced water and this resulted in over a 50%

reduction in permeability. The bacterially produced precipitates alone, he feels, cause 25% of total plugging in the case of consolidated sand cores.

iv) Beneficial activities.

Sulfate-reducing bacteria, as pointed out in the preceding paragraphs, can be damaging to the oil field and its equipment, but the activity of certain strains of Desulfovibrio may be beneficial by increasing recovery in secondary operations. Means by which the bacteria could release oil from petroliferous formations have been enumerated by ZoBell (1947). Some of these are that bacteria will: 1. produce acids which will dissolve calcareous or sulfate minerals increasing porosity and releasing adsorbed fluids, 2. produce gases which increase pressure within the formation, 3. produce detergents which will decrease surface tension resulting in easier flow of fluids, 4. adhere firmly to solids, thus forcing oil off these surfaces, and 5. reduce viscosity of the oil by splitting long hydrocarbon chains. One organism isolated by ZoBell which was thought to have some of the above properties was named Desulfovibrio hydrocarbonoclasticus. Updegraff and Wren (1954) tested 38 different cultures of Desulfovibrio for the beneficial properties mentioned but obtained only negative results. They concluded that currently known strains of sulfate-reducing bacteria can not be applied in the field successfully, yet they do not exclude the possibility that a strain of Desulfovibrio may be found or produced by selective culturing which would be capable of releasing oil from petroliferous formations.

b) Iron bacteria.

i) Description.

A group of microorganisms that deposit iron hydroxide in a "characteristically distinct way" has been referred to as iron bacteria. These organisms have attracted the attention of many workers because of their deposits which are visible to the naked eye. Kützing discovered the first bacteria of this type (Sphaerotilus natans) in 1833. In 1836 Ehrenberg found Gallionella ferruginea, and in 1870 Cohn described Crenothrix polyspora. Winogradsky, who was the first to experiment with cultures of iron bacteria, suggested in 1888 that these microorganisms may utilize energy produced in the process of oxidizing ferrous ions to the ferric state. For this reason all of these organisms were considered to be strict chemoautotrophs until 1910 when Molish succeeded in growing Leptothrix under heterotrophic conditions (Pringsheim, 1949a).

Starkey (1945) has subdivided the iron bacteria into three groups. The first group, Siderocapsa, consists of organisms that occur singly or in irregular aggregates. The second group is made up of bacteria producing filaments. Starkey lists four such genera: Sphaerotilus, Clonothrix, Leptothrix, and Crenothrix. The third group, having one genus, Gallionella, consists of the stalked bacteria. For additional genera proposed, Starkey refers the reader to the monographs of Cholodny (1926) and Dorff (1934).

The characteristic features of Siderocapsa are: the cells are spherical, ellipsoidal, or bacilliform found singly

or in aggregates; some of them produce capsules impregnated with iron or manganese compounds, while others will form iron or manganese precipitates in or on cell surfaces or in the surrounding medium; they are free living, found in surface layers of fresh water or may be attached to submerged objects; some species exhibit motile stages.

The characteristic feature of filamentous iron bacteria is formation of trichomes of various thickness that may be impregnated with iron or manganese compounds. These filaments may show false branching and they may be attached to submerged surfaces. The cells inside the sheath are spherical or cylindrical. Reproduction takes place by non-motile conidia, motile swarmers, individual cells that may slip out of the sheaths or by fragmentation of the filaments.

Gallionella is one of the most interesting of the iron bacteria because of its characteristically twisted stalk. The filament was described by Ehrenberg in 1836 who called it the organism with a "rosary form". Griffith (1853) pointed out that this appearance was due to two filaments that were interwoven forming a spiral which may be mistaken for a bead-like structure. Reproduction was thought to take place either by simple fission or conidia that appeared on the sides of filaments as described by Ellis (1919). In 1926 Cholodny reported observing a bean-shaped bacillus at the end of the filament which seemed to secrete the stalk. From that time on the filament has been considered to be an inorganic product of the cell's metabolism. The twisting of

the ribbon was explained as being due to torsional action exerted by the cell. Some workers doubted that the filaments were completely inorganic (Suessenguth, 1927). Butkewitsch (1928) believed that Gallionella threads are capable of independent growth on the ground that they produce "buds" and "conidia" which were observed by Ellis (1919).

Van Iterson (1958) made a thorough study of Gallionella cultures and her report is accompanied by excellent electron micrographs. From these it is evident that the stalk is not homogeneous ferric hydroxide but consists of many individual strands which are impregnated with ferric hydroxide. She was able to show that the core of these strands is organic by fixing it in the electron beam and then chemically removing the iron hydroxide. She also observed "dark knobs" at the ends of certain strands which she interpreted as suggesting growth of these strands. Van Iterson further suggested that oxidation occurs within the threads and that the released energy is used for synthesising new strand material. Van Iterson seldom found the terminal cells reported by Cholodny, however she observed "side cells" more frequently. Other cells observed were "spirillum-like", "egg-shaped", and distorted forms. She also reported presence of "bladder-like structures" that contain bodies which are smaller than one micron. A very fascinating phenomenon observed by van Iterson is that Seitz or collodion filtrates of Gallionella cultures produced typical filaments when inoculated into Lieske's medium. This development is

explained as being due to either "symplasm" or "primordial plasm".

Iron bacteria are normal inhabitants of waters containing appreciable amounts of dissolved iron. They are found in springs, wells and streams, as well as marshes and lagoons. By oxidizing ferrous compounds to the ferric state, they obtain energy for their metabolic processes. This reaction has been expressed by Silverman and Lundgren (1959) as follows:

$$4FeSO_4 + O_2 + 10H_2O \longrightarrow 4\,Fe(OH)_3 + 4\,H_2SO_4$$

Strict autotrophs e.g. Ferrobacillus ferrooxidans depend entirely on this reaction for their energy while facultative autotrophs e.g. Sphaerotilus natans will also grow on organic media. Harrison and Heukelikian (1958) reported that Sphaerotilus natans will use various compounds as a carbon source, such as mono- and disaccharides, organic acids, alcohols, and amino acids. Gallionella ferruginea has been until recently regarded as a strict autotroph, but van Iterson (1958) succeeded in growing it on nutrient agar. Under those circumstances only cellular multiplication occurs without production of the typical stalks.

Under heterotrophic conditions, it has been shown, that Sphaerotilus natans will utilize organic compounds as its nitrogen source. Inorganic nitrogen in the form of nitrate may also be used. Ammonium utilization depends on the type of carbon source available but excess of ammonia appears inhibitory (Harrison and Heukelekian, 1958). In the culture media used for growing Gallionella ferruginea (van Iterson,

1958) and Ferrobacillus ferrooxidans (Silverman and Lundgren, 1959) ammonium salts were used as the sole source of nitrogen. Lieske (1911) incorporated ammonium and nitrate compounds in his culture medium for Gallionella.

The temperature range of iron bacteria is from 0°C to about 40°C. Lieske (1911) observed only Gallionella stalks in a brook during the winter months and mostly Leptothrix with traces of Gallionella during the summer. In artificial cultures he recorded growth between 0° and 22°C with optimum growth at 6°C. Wolfe (1958) and van Iterson (1958) kept their cultures of Gallionella at room temperatures and reported good growth. The temperature range of filamentous iron bacteria (e.g. Sphaerotilus) is between 15° and 40°C with the optimum at 30°C according to Stokes (1954). Höhnl (1955) obtained maximum growth with a wild strain at 10°C and 15°C with a cultured strain. Harrison and Heukelekian (1958) feel that the temperature effect, as such, has been overemphasized. The low temperatures that seem to favour growth of Sphaerotilus could be due to the fact that it influences other factors such as nutrition and oxygen supply. Van Iterson believes that the low temperature effect is due to a direct influence on ferrous ion concentration. Leptothrix thermalis and Siderobacter thermalis, appear to be thermophilic for they were found at 50°C (van Iterson).

Iron bacteria, as a group, are active in a wide range of hydrogen ion concentrations. At the lower extreme we find Ferrobacillus ferrooxidans which grows well at a pH of 2.0

to 3.0. The optimum pH for Gallionella in culture is reported by Wolfe as being 6.6. Growth of Sphaerotilus has been observed between pH 6 and 10 (Harrison and Heukelekian, 1958), while Siderocapsa was found only in alkaline hard water and never in neutral or acid soft waters (Hardman and Henrici, 1939).

The iron bacteria are aerobic and thus depend on the presence of free oxygen for respiration. The oxidation of ferrous compounds to ferric compounds supplies energy to these organisms. However, in this regard, they have to compete with gaseous oxygen which if present at a high enough concentration will cause the ferrous compounds to oxidize spontaneously to ferric hydroxide. Thus, for the bacterial enzymes to compete against spontaneous oxidation the oxygen tension must be at a favourable level. Van Iterson (1958) showed this effect by keeping Gallionella cultures in atmospheres of different oxygen concentration. Gaufin and Tarzwell (1956) reported growth of Sphaerotilus in a creek with 0.5 ppm of dissolved oxygen. Stokes (1954) found growth of S. natans under conditions which were considered anaerobic but, apparently, had traces of oxygen.

Light seems to have no influence on the development of iron bacteria because they are found growing in open and shady places, as well as in absolute darkness (Starkey, 1945). No actual experiments have been done to test this factor.

The presence of dissolved iron is essential for iron bacteria but their tolerance of iron concentration is very wide. Schorler (1906) reported the presence of deposits of

iron bacteria in pipes carrying water with 0.2 ppm iron. This could be accounted for by the constant fresh supply of the substrate. Iron bacteria were also reported present in spring water containing 1 to 10 ppm of iron (Halvorson, 1931). Van Iterson isolated Gallionella from ditch water containing 6 ppm of iron. The amount of dissolved iron would undoubtedly be controlled by pH, oxygen tension, and organic matter present. Furthermore, organic matter itself could be a source of iron upon its decomposition (van Iterson, 1958).

ii) Adverse activities.

The ability of iron bacteria to cause an accumulation of ferric hydroxide which is many times greater than the cell material makes them most important as fouling organisms. They cause hard deposits or accumulations of filamentous slimy material on pipe walls thus decreasing the water carrying capacity. Schorler (1906) reported that a pipe with a 10 cm diameter showed a deposit of ferric hydroxide 3 cm thick on its walls leaving an opening of only 4 cm diameter. Iron bacteria also cause discoloration and turbidity of water and may be indirectly responsible for a bad taste. Iron bacteria have been found to cause trouble in waters coming from deep wells (Carrol, 1941) and organic soils (Tenny, 1939) containing iron or manganese compounds. They have also been troublesome in surface waters where iron compounds were present originally in the water or accumulated from the dissolution of iron containers. Larson (1939) showed that water leaving a water-treatment plant iron free and subsequently flowing through iron pipes may have as much as

8 ppm only a short distance away. Alexander (1944) warns that treated water at the plant containing no fouling organisms will not necessarily remain so throughout the whole system.

Merkt (1943) showed that iron bacteria caused 75% plugging of limestone cores and about 20% of unconsolidated sand cores. In the first instance the iron hydroxide filaments did not penetrate the core to any extent, while in the second instance the filamentous material was found throughout the entire core.

Filamentous iron bacteria (Sphaerotilus) have also been held responsible for slime infestation in streams (Harrison and Heukelekian, 1958).

The iron bacteria are mainly fouling organisms and do not cause corrosion. In cases where corrosion has been reported in presence of iron bacteria it is believed to be an indirect effect (Starkey, 1945).

c) Slime formers.

The outer surface of the bacterial cell consists of a layer of slime. The thickness of this layer varies with the species and within the species and is influenced by environmental conditions. Certain bacteria constantly form a thick layer of slime which is called a capsule. The ability to produce slime is therefore not the characteristic of just a few organisms but of most bacteria. Factors which were found to influence capsule formation in some organisms are temperature, nutrients, and concentrations of electrolytes (Strandskov, 1948). In the majority of organisms the capsular material is polysaccharide in nature, less frequently polypeptides are found. Yet, not all strains of a given capsule-producing bacterium have a mucilagenous material of the same chemical composition (Stanier, Doudoroff, and Adelberg, 1957).

The presence of slime can be very troublesome in industrial processes, e.g. cooling systems, filtration, etc. Slime is damaging in flooding operations for secondary oil recovery since the slime plus cell aggregates will plug larger pores or a greater area of a fine porous surface than single bacterial cells.

MATERIALS AND METHODS

MATERIALS AND METHODS.

1. Source of Water.

The principal oil field under study is the Pembina field, one of the largest in North America. It was discovered in 1953 some 70 miles west-southwest of Alberta's capital, Edmonton. This oil field covers an area of 400,000 acres and contained originally 3.19 billion barrels of oil (Justin, 1957). Previous to flooding 49 million barrels of oil were produced (Oil and Gas Conservation Board, 1960a). The first water flooding operation was started in 1956. At present there are 613 injection wells (Oil and Gas Conservation Board, 1960b) injecting fresh water at an average rate of 300 barrels of water per day. Water used for flooding operations is mostly surface water, water from shallow wells and deep wells (up to 600'). A new method of obtaining filtered water from underneath a river bed has been developed. To a small extent produced water is used. Surface water and produced water are invariably treated before being used for injection. The wells yield water with low salt content and are thus regarded as containing "fresh water". The treatment of well water, if any, is rather superficial. The water for flooding purposes is often stored in steel storage tanks but in the case of water coming from a deep well it is often pumped directly into an injection well within a few yards of the water well.

All samples of injection water analysed in our laboratory were collected by the operators who were instructed as to the proper methods of collection (clean

sterile screw-capped bottles of 100 ml capacity were used for all sampling). The operators were asked to obtain samples which were truly representative of the water supply. The water sample was delivered to our laboratory within 24 hours of sampling and if the time lapse between sampling and delivery was longer than four hours the bottles were kept in an ice box or refrigerated at 7°C.

Methods of investigation are based on recommendations given by the American Petroleum Institute (1958). These involve aerobic and anaerobic viable counts of bacteria, use of special media to detect fungi and sulfate reducing bacteria, and preparation of microscopic slides to detect iron bacteria, algae, and diatoms.

2. Microscopic and Cytological Technique.

a) Immediate observations.

Fifty milliliters of the water specimen is centrifuged at 2,500 to 3,000 R.P.M. for 30 minutes. The supernatant is decanted and a wet mount of the centrifugate prepared by placing a loopful of the material on a clean glass slide. This drop is covered with a cover-slip and the preparation observed through a microscope having conventional light and phase contrast. For this purpose a Reichert microscope is used at magnifications of 900 times or less. In particular the presence or absence of algae and diatoms is noted and recorded. If large numbers of these organisms are present this is indicated on the report form (Appendix I).

b) Staining technique for iron-depositing bacteria.

Two slides are prepared by allowing about one drop of the sediment material (see above) to air dry on clean slides. The film is then fixed to the glass surface with methyl alcohol. One of the slides is immersed in a mixture of hot potassium ferrocyanide and acetic acid and the other in a mixture of hot potassium ferricyanide and acetic acid for two minutes. If a ferric compound is present on the slide it will react with potassium ferrocyanide forming Prussian blue (ferric ferrocyanide) while a ferrous compound will react with potassium ferricyanide producing Turnbull's blue (ferro ferricyanide). After washing the slides in distilled water safranin is used as a counterstain which stains organic matter (Myers, 1958a). The smears are washed and examined under the oil immersion objective (magnification

900x) and the presence or absence of iron bacteria recorded.

3. Method for Detection of Organisms.

a) Sulfate-reducing bacteria.

The number of Desulfovibrio present in the injection waters was obtained by using a special medium ("SR-medium"). It was developed in the Department of Bacteriology of the University of Alberta on the basis of Allred's medium (Allred et al., 1954) and one used by Armour Chemical Division (1958). This medium contains sodium lactate, 0.7%; yeast extract, 0.1%; ascorbic acid, 0.01%; $MgSO_4$, 0.02%; K_2HPO_4, 0.05%; $Fe(NH_4)_2(SO_4)_2$, 0.01%; $FeSO_4$, 0.01%; sodium thioglycolate, 0.01%; NaCl, 1.0%; and agar, 1.5%. The pH of the medium is adjusted to 7.2 with one normal NaOH. It is dispensed in 9 ml quantities in 4 by 5/8 inch screw-capped test tubes and in 20 ml quantities in 8 by 1 inch test tubes. After sterilization, the tubes are cooled to 45°C in a water bath. The large test tube is inoculated with 10 ml of the water sample and the four small tubes are inoculated with 1 ml portions of the undiluted material as well as 1 ml of 1:10, 1:100, and 1:1,000 dilutions. The tubes are allowed to cool rapidly and incubated at 20 to 25°C. Colony counts are made after 7 and 21 days incubation and recorded on the report sheet as organisms per ml or per 10 ml. The colonies of sulfate reducers are recognized as black colonies in the deep agar tubes due to the formation of the black iron sulfide by the reaction of the evolving H_2S with the iron salts in the medium.

A preliminary experiment was done to determine the

best method of obtaining serial dilution of the injection water. For this purpose the SR-medium itself was used after it was melted and then cooled to 45°C. For comparison sterile distilled water blanks were used and also sterile distilled water containing 0.01% ascorbic acid and 0.01% sodium thioglycollate. As an inoculum a culture of Desulfovibrio which had been isolated from injection water was used. The melted SR-medium was noticed not to be a good diluting fluid due to its high viscosity. The mixture of ascorbic acid and sodium thioglycollate (freshly prepared) was found to be superior as a diluting fluid in that it gives counts of sulfate-reducing bacteria ten times higher than when ordinary distilled water is used.

b) Aerobes and anaerobes.

Tryptone Glucose Yeast Agar (Difco) is the medium used for making viable bacterial count. Dilutions of the water specimen and pour plates are made in the manner prescribed by the tenth edition of "Standard Methods for the Examination of Water, Sewage and Industrial Wastes". Cultures are incubated under aerobic and anaerobic conditions at 20° to 25°C. After three and seven days incubation the colonies are counted and the number of organisms per 1.0 ml of water sample calculated. Results are recorded in the report sheet.

c) Slime formers.

i) Non-spore-forming organisms.

A 0.1 ml sample of the water is spread evenly over the surface of Tryptone Glucose Yeast Agar (Difco) with a sterile spreader. The medium is poured into the Petri plates immediately before using and is used only on the day it is prepared. The inoculated plates are incubated at 20° to 25°C and the presence of mucoid colonies determined after three and seven days incubation.

ii) Spore-forming organisms.

A ten milliliter portion of the sample is transferred to a sterile test tube using a sterile pipette. The tube and contents are then placed in an 80°C water bath and maintained at that temperature for fifteen minutes. A 0.1 ml portion of the heated specimen is used to inoculate the surface of Tryptone Glucose Yeast Agar (Difco) medium and the same procedure followed as in Section c) i).

d) Actinomycetes.

The culture prepared in Section c) i) for the detection of slime forming bacteria is also examined after three and seven days incubation for the presence of Actinomycetes. Suspected colonies are smeared, stained by Gram's procedure, and examined under the microscope. Observations are recorded on the report sheet.

e) Fungi.

A 0.1 ml sample of the water is inoculated on the surface of Sabouraud's Agar medium (Difco) and spread evenly with a sterile spreader. The inoculated plate is incubated at 20° to 25°C and the presence of yeast and mold colonies noted after three and seven days incubation. To confirm the presence of yeasts, suspected colonies are smeared, stained, and observed with a Reichert microscope using the 1.25 mm objective.

RESULTS

RESULTS

1. Specificity of Iron Stain.

Bacteria belonging to the genus Siderocapsa, where ferric compounds are deposited in or on cell surfaces, appear blue under the microscope when exposed to hot potassium ferrocyanide mixture (see "Staining technique" page 25). The Prussian blue reaction is often sufficiently intense that the counterstain staining the cell cannot be noticed. It would have been very interesting to use the described staining procedure to stain a species of Siderocapsa which produces capsules impregnated with ferric compounds. This was not done because a culture of such organisms was not available.

The trichomes of filamentous bacteria, when exposed to potassium ferrocyanide, acquired stronger blue coloration than when brought into contact with potassium ferricyanide. This indicates that the majority of the iron in the trichomes is in the ferric state. The sheaths are sometimes empty but when bacilli are present these will be stained pink with safranin (see Figure 1, page 35).

The Gallionella stalks generally appear blue when exposed to potassium ferrocyanide. Yet, frequently, it was observed that they were negative to both Prussian blue and Turnbull's blue reactions. This would suggest that they might use other metals besides iron to obtain required energy, possible manganese. "End cells" described by Cholodny or "side cells" described by van Iterson were never observed in this study.

The method for staining iron bacteria was studied using

Figure 1.

Trichomes of iron bacteria with cells (1,750 X).

all the cultures in the stock of the Department of Bacteriology. These are listed in Appendix II. All bacteria were cultured in Nutrient Broth (Difco) except Corynebacterium, Micrococcus, and Streptococcus which were grown in serum broth (Nutrient Broth (Difco) containing 5% human serum). All cultures were incubated at 37°C for a period of 24 hours and smears of this material were prepared by allowing one drop of the culture to air dry on a clean slide. The staining procedure was carried out as outlined on page 25.

Examination of the slides revealed that bacteria stained with potassium ferricyanide acquired a bluish tinge. This indicates that all the organisms studied contain a small amount of iron which is in the ferrous state and the Turnbull's blue, thus formed, either masks or interferes with the staining by safranin. Apart from the bacteria blue stained granular material was observed. The presence of this material cannot be attributed to bacterial action because identical blue granules were seen on "control" slides where sterile medium was used to prepare the smear.

Bacteria stained with potassium ferrocyanide and counterstained with safranin were all bright pink indicating the absence of ferric ions. The smear itself revealed no blue material but instead green crystals were seen. These crystals are formed when the mixture of potassium ferrocyanide and acetic acid are heated to about 70°C into which the slides are submerged. Sometimes the crystals are hard to wash off

the slides without damaging the smear.

Some fungi (16 cultures in all) were also subjected to the above stain (see Appendix II). These organisms were grown in Sabouraud Liquid Medium (Difco) at room temperature for four days. The smears were then prepared and stained as previously outlined. In all cases where potassium ferricyanide was used the filaments of the mold were stained faint blue and pink in patches. The yeast cells were affected by the stain in the same way as bacterial cells described above. Appearance of blue granular material and green crystals on slides was the same as in the case of bacterial cultures.

2. Specificity of SR-medium.

A typical colony of sulfate-reducing bacteria as it appears in SR-medium after 21 days of incubation is shown in Figure 2, page 39. Four tubes were inoculated with injection water as outlined under "Methods for Detection of Organisms", page 27. The colonies frequently appear before seven days of incubation as small black dots, but sometimes they do not develop until after fourteen days of incubation. Since no new colonies were ever observed to develop after three weeks of incubation our present routine is to keep all cultures for 21 days. Growth of the colonies can be observed as they enlarge and the area of black iron sulfide deposit increases immediately surrounding the colony leaving the remainder of the medium unaffected.

To test the effect of non-sulfate-reducing bacteria on SR-medium all the genera and species of microorganisms in stock in the Department of Bacteriology were used to inoculate the above medium. These are listed in Appendix III. All the bacteria were maintained as stock cultures in Nutrient Broth (Difco) except the Clostridium species which were grown in cooked-meat medium (Mackie and McCartney, 1948), and Corynebacterium, Micrococcus and Streptococcus, which were grown in serum broth. Prior to testing, the cultures were transfered to fresh medium and incubated at 37°C for 24 hours. The fungi used in this experiment were maintained in Sabouraud Liquid Medium (Difco) and incubated at room temperature. Four day old cultures were used for inoculation

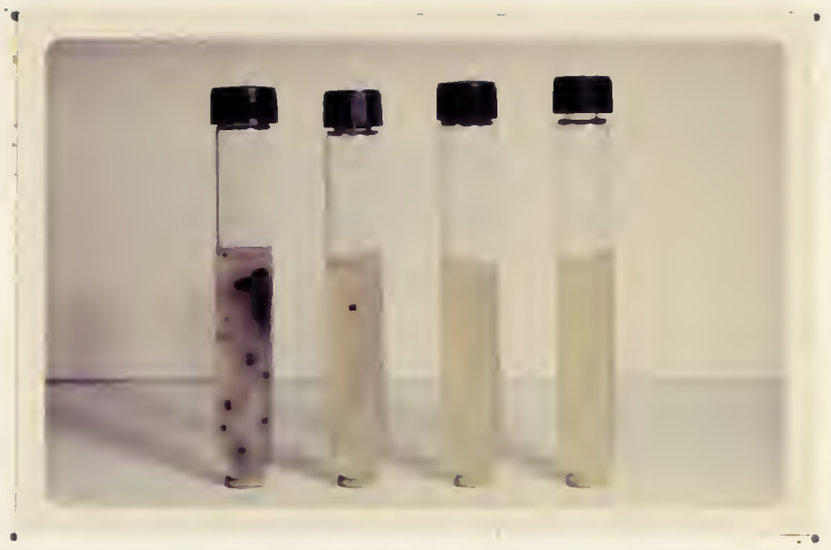

Figure 2.

SR-medium inoculated with injection water (No. 270) after 6 days incubation showing colonies of Desulfovibrio.

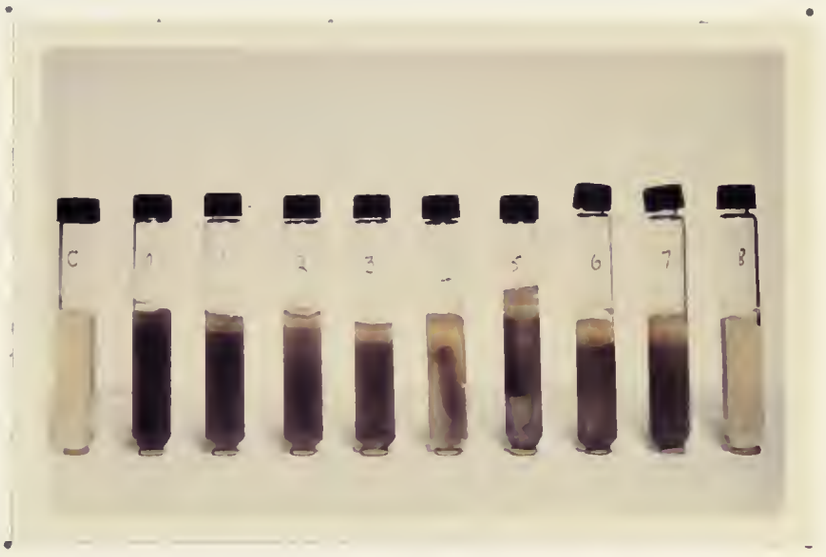

Figure 3.

SR-medium inoculated with a culture of Proteus vulgaris after 21 days of incubation.

of the medium under study.

After seven days of incubation at room temperature it was noticed that the medium inoculated with Proteus mirabilis, P. morganii, P. vulgaris, Salmonella choleraesuis, S. oranienburg, S. paratyphi A, S. paratyphi C, and S. schottmuelleri turned grey. All the other organisms had no effect on the color of the medium. These observations did not change even after 21 days of incubation.

Since the degree of dilution of the cultures was not great enough to produce well isolated single colonies in any of the tests with Proteus or Salmonella it was decided to repeat the experiment with representative cultures of these genera. Proteus vulgaris and Salmonella paratyphi A cultures were selected for this purpose. After seven days of incubation both cultures showed H_2S production throughout the tubes containing higher concentration of microorganisms. Individual colonies of S. paratyphi A appeared in higher dilution after fourteen days of incubation. These were observed to be small and yellow in color. Only after 21 days of incubation did they acquire a black appearance, leaving the remainder of the medium unaffected. Such individual colonies were not observed in the case of Proteus vulgaris (see Figure 3, page 39).

3. Tables of Results.

Data concerning all water samples tested are tabulated according to the source of the sample. Water used as the source for flooding operation has been subdivided into "well water" and "surface water". Well water includes water coming from shallow wells and deep wells ranging in depth up to 600 feet. Surface water includes water obtained from streams, rivers, or lakes. Water coming from wells had low salt content and is thus regarded as 'fresh water'. In all the cases the source water was not treated before it was sampled for microbiological analysis. Where the temperature of the water source and the depth of the well were known tables have been constructed showing these factors.

The rest of the water samples analysed are listed in tables headed: "Storage tanks", "Pipes, pumps, etc.", "Injection well head", and "Produced water" according to the location of the sampling point.

Most of the water that has been sampled at the injection well head, it is known, has been subjected to one or another type of treatment. This could be either filtration, sedimentation, deaeration, chlorination, addition of bactericides, or any combination of these treatments. Incomplete information regarding the exact method of treatment for individual samples prevents classification on this basis.

Tables I to VI give the detailed results obtained in the microbiological examination of 287 water samples studied in this investigation. Tables VII to X present

data in a more condensed form. Tables XI to XIII have been constructed in an attempt to show correlation between environmental factors and types of organisms present as well as their numbers. Actual counts of viable bacteria obtained are grouped into classes and presented in Tables XIV to XVIII. Table XIX gives the frequency of occurrence and the genus of iron bacteria noted. Table XX gives the actual numbers of viable bacteria obtained after various incubation periods, while Table XXI summarizes this information.

TABLES

TABLE I

Microbiological Analysis of Injection Water (Source: Wells).

Lab. no.	Molds	Yeasts	Algae	Diatoms	Actino-mycetes	Slime formers		sulfate-reducing bacteria per ml	Iron bacteria	Aerobic viable count/ml	Anaerobic viable count/ml
						non-sporing	sporing				
81	-	-	-	-	-	-	-	2	-	19,000	7
82	-	-	-	-	-	present	-	-	-	112,000	78
83	present	-	-	-	-	-	-	-	-	2,000	0
84	-	-	-	-	-	present	-	-	-	3,000	3
85	present	-	-	-	-	-	-	-	-	5,000	1
86	present	-	-	-	-	-	-	-	-	1,000	1
121	-	-	-	-	-	-	-	2	-	7,000	25,000
124	-	present	-	-	-	-	-	10	-	390	0
146	-	-	-	-	-	-	-	-	-	10,000	0
149	-	-	-	-	-	present	-	-	-	1,000	0
153	-	present	-	-	-	-	-	210	-	2,200	670
154	-	-	-	-	-	-	-	1,000	-	83,000	25
160	-	-	-	-	-	present	-	70	-	1,700	42
161	-	-	-	-	-	present	-	3	-	1,200	0
162	-	-	-	-	-	present	-	-	-	300	0
220	-	-	-	-	-	-	-	20	-	5,000	100
225	present	present	-	-	-	present	-	10	-	500,000	16,000
235	-	-	-	-	-	-	-	-	-	150,000	5
259	-	-	-	-	-	-	-	-	-	50	2
275	-	-	-	-	-	-	-	-	Ga.*	3,000	1

TABLE I (Continued)

Lab. no.	Molds	Yeasts	Algae	Diatoms	Actino-mycetes	Slime formers: non-sporing	Slime formers: sporing	sulfate-reducing bacteria per ml	Iron bacteria	Aerobic viable count/ml	Anaerobic viable count/ml
282	-	-	-	-	-	-	-	-	-	200	0
283	-	-	-	-	-	-	-	-	-	300	0
284	-	-	-	present	-	-	-	-	-	150	0
285	-	-	-	-	-	present	-	2	-	330,000	8
302	-	-	-	-	-	-	-	-	Ga.	450,000	8,000
303	-	-	-	-	-	-	-	60	-	2,100,000	50,000
304	-	-	-	-	-	-	-	-	Ga.	350,000	800
314	-	-	-	-	-	-	-	60	-	15,000	10
315	-	-	-	-	-	-	-	<1	-	4,500	7
316	-	-	-	-	-	-	-	100	-	80,000	400
317	present	-	-	-	-	-	-	60	-	300,000	20
330	-	-	-	-	-	-	-	-	-	4,000	0

* Cr. = Crenothrix
Ga. = Gallionella
Si. = Siderocapsa
Sph. = Sphaerotilus

TABLE II

Microbiological Analysis of Injection Water (Source: Lakes and Rivers).

Lab. no.	Molds	Yeasts	Algae	Diatoms	Actino-mycetes	Slime formers		sulfate-reducing bacteria per ml	Iron bacteria	Aerobic viable count/ml	Anaerobic viable count/ml
						non-sporing	sporing				
39	-	-	-	-	-	-	-	30	-	1,800	72
43	-	-	-	-	-	-	-	-	-	113	0
44	-	-	-	-	-	-	-	-	-	110	0
69	-	-	-	present	-	present	-	-	-	190,000	37
73	-	-	-	-	-	present	-	<1	-	9,000	70
74	-	-	-	-	-	present	-	2	-	750	30
75	-	-	-	-	-	present	-	-	-	1,000	35
76	-	-	-	-	-	present	-	-	-	260	16
77	-	-	-	-	-	-	-	-	-	230	10
113	-	-	present	present	-	present	-	-	-	12,000	490
127	-	-	-	present	-	present	-	<1	-	1,600,000	0
159	-	-	-	-	-	present	-	2	-	800	0
305	-	-	-	-	-	-	-	-	-	5,000	5,000
306	-	-	-	-	-	present	-	-	-	1,700	100
334	present	-	-	present	-	present	-	2	-	36,000	200

TABLE III

Microbiological Analysis of Injection Water (Source: Storage Tanks).

Lab. no.	Molds	Yeasts	Algae	Diatoms	Actino-mycetes	Slime formers: non-sporing	Slime formers: sporing	sulfate-reducing bacteria per ml	Iron bacteria	Aerobic viable count/ml	Anaerobic viable count/ml
6	-	-	-	-	-	-	present	40,000	-	17	0
7	-	-	-	-	-	-	-	600	-	120	250
128	-	-	-	-	-	-	-	TNTC**	Si.*	0	0
165	-	-	-	-	-	present	present	TNTC	-	2,500,000	3,300
169	-	-	-	-	-	-	-	20,000	-	1,000,000	500
216	-	-	-	-	-	-	-	2	Sph.	100,000	30
226	-	-	-	-	-	-	-	10	-	0	0
261	-	-	-	-	-	-	-	-	-	460	50
277	-	-	-	-	-	-	-	-	-	4,000	0
286	-	-	-	-	-	-	-	3	-	25	0
287	-	-	-	-	-	-	-	20	-	800	400
319	present	-	-	-	-	present	-	2	-	28,000	0
320	-	-	-	-	-	-	-	15	-	300,000	1,000
331	present	-	-	-	-	present	-	-	-	5,200	4

* Cr. = Crenothrix
Ga. = Gallionella
Si. = Siderocapsa
Sph. = Sphaerotilus

** Too numerous to count.

TABLE IV

Microbiological Analysis of Injection Water (Source: Pipes, Pumps, etc.).

Lab. no.	Molds	Yeasts	Algae	Diatoms	Actino-mycetes	Slime formers: non-sporing	Slime formers: sporing	sulfate-reducing bacteria per ml	Iron bacteria	Aerobic viable count/ml	Anaerobic viable count/ml
9	-	-	-	-	-	-	-	-	-	300	8
45	-	-	-	-	-	-	-	-	-	1,400	5
46	-	-	-	-	-	-	-	-	-	1,300	3
102	present	-	-	-	-	-	-	-	-	200	0
120	-	-	-	-	-	-	-	-	Ga. + Sph.*	20,000	120
122	present	-	-	-	-	present	-	2	-	106,000	200
126	-	-	-	-	-	-	-	<1	Sph.	300,000	0
133	-	-	-	-	-	-	-	-	-	1,800	0
134	-	-	-	-	-	-	-	-	-	220	16
212	-	-	-	-	-	present	-	-	-	1,000	100
217	-	-	-	-	-	-	-	2	-	45,000	10
218	-	-	-	-	-	present	-	<1	-	40,000	200
219	present	-	-	-	-	-	-	2	-	90,000	40
233	present	-	-	present	-	-	present	1	-	73,000	1,500
252	present	-	-	present	-	present	-	<1	-	15,500	200
273	-	-	-	-	-	-	-	15	-	300,000	5,000
274	-	-	-	-	-	-	-	6	-	400,000	3,000
276	present	-	-	-	-	-	-	<1	-	42,000	2
278	-	-	-	-	-	-	-	-	-	16,000	50
280	present	-	-	-	-	-	-	-	-	12,000	150
288	-	-	-	-	-	-	-	-	-	600	5
289	-	-	-	-	-	-	-	-	-	30,000	800
290	-	-	-	-	-	-	-	-	-	32,000	10,000
291	-	-	-	-	-	-	-	-	-	12,000	2,000
321	-	-	-	-	-	-	-	-	-	500,000	15

TABLE IV (Continued)

Lab. no.	Molds	Yeasts	Algae	Diatoms	Actino-mycetes	Slime formers		sulfate-reducing bacteria per ml	Iron bacteria	Aerobic viable count/ml	Anaerobic viable count/ml
						non-sporing	sporing				
323	-	-	-	-	-	-	-	2	-	1,000	6
324	-	-	-	-	-	-	-	-	Ga.	6	0
327	-	-	-	-	-	-	-	-	-	0	0
328	-	-	-	-	-	-	-	-	-	0	0
329	present	-	-	-	-	present	-	10	-	20,000	10
343	-	-	-	-	-	-	-	-	-	2,000	20
344	-	-	-	-	-	present	-	-	-	3,800	0
345	present	-	-	-	-	present	-	-	-	50,000	0
346	-	-	-	-	-	-	-	-	-	20	0
347	-	-	-	present	-	-	-	-	-	120,000	0
348	-	-	-	-	-	-	-	10	-	2,500	20
349	-	-	-	-	-	-	-	30	-	2,500	10
350	present	-	-	-	-	-	-	-	-	4,000	0

*
Cr. = Crenothrix
Ga. = Gallionella
Si. = Siderocapsa
Sph. = Sphaerotilus

TABLE V

Microbiological Analysis of Injection Water (Source: Injection Well Head).

Lab. no.	Molds	Yeasts	Algae	Diatoms	Actino-mycetes	Slime formers: non-sporing	Slime formers: sporing	sulfate-reducing bacteria per ml	Iron bacteria	Aerobic viable count/ml	Anaerobic viable count/ml
1	-	-	-	-	-	-	-	3	-	220,000	0
2	-	-	-	-	-	-	-	<1	-	160,000	0
3	-	-	-	-	-	-	-	<1	-	80,000	0
4	-	-	-	-	-	-	-	<1	-	130,000	20
5	present	-	-	-	-	-	-	<1	-	100,000	10
10	-	-	-	-	-	-	-	10	-	10,000	6
11	-	-	-	-	-	-	-	1,000	-	125,000	50
12	-	-	-	-	-	-	-	-	-	1,700	12
13	present	-	-	-	-	-	-	-	Ga.*	550	0
14	-	-	-	-	-	-	-	-	-	500	0
15	-	-	-	-	-	-	-	-	-	940	20
16	-	-	-	-	-	-	-	-	-	16,000	0
17	-	-	-	-	-	-	-	-	-	45,000	11
18	present	-	-	-	-	-	-	-	-	550	5
19	-	-	-	-	-	-	-	-	-	4,900	0
20	-	-	-	-	-	-	-	-	Ga.	8,400	0
21	-	-	-	-	-	-	-	5	-	4,000	120
22	-	-	-	-	-	-	-	-	Sph.+ Ga.	4,800	15
23	-	-	-	-	-	-	-	13	-	85	0
24	-	-	-	-	present	-	-	6	-	460	15
25	-	-	-	-	-	-	-	-	-	0	0
26	-	-	-	-	-	-	-	-	-	740	0
27	-	-	-	-	-	-	-	-	-	16	0
28	-	-	-	-	-	-	-	-	-	200,000	500
29	-	-	-	-	-	-	-	2	-	100,000	80

TABLE V (Continued)

Lab. no.	Molds	Yeasts	Algae	Diatoms	Actino-mycetes	Slime formers: non-sporing	Slime formers: sporing	sulfate-reducing bacteria per ml	Iron bacteria	Aerobic viable count/ml	Anaerobic viable count/ml
30	-	-	-	-	-	-	-	-	-	0	0
31	-	-	-	-	-	-	-	-	-	330	0
32	-	-	-	-	-	-	-	-	-	4,500	0
49	present	-	-	-	-	-	-	15	-	9,000	200
50	present	-	-	-	-	-	-	-	-	470	0
51	present	-	-	-	-	-	-	20	-	0	0
52	-	-	-	-	-	-	-	-	-	10	0
53	-	-	-	-	-	-	-	1	-	4,200	5
54	-	-	-	-	-	-	-	-	-	150	4
55	-	-	-	-	-	-	-	-	-	160	12
56	present	-	-	-	-	-	-	-	-	12	2
57	-	-	-	-	-	-	-	8	-	10,000	120
58	present	-	-	-	-	-	-	-	-	0	0
59	present	-	-	-	-	-	-	-	-	340	0
60	-	-	-	-	-	-	-	-	-	6	0
61	-	-	-	-	-	-	-	-	-	0	9
62	-	-	-	-	-	-	-	-	-	3,500	0
63	-	-	-	-	-	-	-	-	-	0	0
64	-	-	-	-	-	-	-	4	Ga. + Sph.	3,000	16
65	-	-	-	-	-	-	-	1	Ga.	120	0
88	present	-	-	-	-	present	-	<1	-	10,800	0
89	-	-	-	-	-	-	-	10	Ga.	1,400	1
90	-	-	-	-	-	-	-	-	-	12,000	1
91	-	-	-	-	-	present	-	-	-	1,000	0
92	present	-	-	-	-	-	-	3	-	61,000	0
93	-	-	-	-	-	present	-	-	-	800	9
94	present	-	-	-	-	present	-	-	-	20,000	0

TABLE V (Continued)

Lab. no.	Molds	Yeasts	Algae	Diatoms	Actino-mycetes	Slime formers: non-sporing	Slime formers: sporing	sulfate-reducing bacteria per ml	Iron bacteria	Aerobic viable count/ml	Anaerobic viable count/ml
95	present	-	-	-	-	-	-	-	-	101,000	0
96	present	-	-	-	-	present	-	-	-	31,000	0
97	-	-	-	-	-	present	-	-	-	99,000	1
99	present	-	-	-	-	present	-	-	-	900	1
103	present	-	-	-	-	-	-	-	-	800	0
105	-	-	-	-	-	-	-	-	-	400	0
106	-	-	-	-	-	-	-	-	-	46,000	0
107	-	-	-	-	-	-	-	-	-	200	0
108	present	-	-	-	-	-	-	-	-	200	0
109	present	-	-	-	-	-	-	-	Ga.	1,300	0
110	present	-	-	-	-	-	-	-	-	900	0
111	-	-	-	-	-	-	-	-	Ga.	1,100	0
112	-	-	-	-	-	-	-	-	-	13,000	0
114	-	-	-	-	-	-	-	-	-	12,000	20
115	-	-	-	-	-	present	-	-	Sph. + Ga.	200	0
123	-	-	-	-	-	present	-	3	-	107,000	3
129	-	-	-	-	-	-	-	-	-	0	0
130	-	-	-	-	-	-	-	-	-	3,800	19
131	-	-	-	-	-	present	-	<1	-	21,200	18,500
136	-	-	-	-	-	-	-	-	-	1,460	250
138	-	-	-	-	-	-	-	-	-	11	3
140	-	-	-	-	-	-	-	-	-	0	0
141	-	-	-	-	-	-	-	23	-	56,500	0
142	-	-	-	-	-	-	-	-	-	200	3
152	-	-	-	-	-	-	-	5	-	1,000	0
170	-	-	-	-	-	-	present	-	-	800	0
171	-	-	-	-	-	-	-	-	-	30	0

TABLE V (Continued)

Lab. no.	Molds	Yeasts	Algae	Diatoms	Actino-mycetes	Slime formers: non-sporing	Slime formers: sporing	sulfate-reducing bacteria per ml	Iron bacteria	Aerobic viable count/ml	Anaerobic viable count/ml
172	-	-	-	-	-	present	-	-	-	400	0
173	-	-	-	-	-	present	-	-	-	500	0
174	-	-	-	-	-	-	-	-	-	800	0
175	-	-	-	-	-	-	-	-	-	300	0
176	-	-	-	-	-	-	-	1	-	250	8
177	-	-	-	-	-	-	-	-	-	20	0
178	-	-	-	-	-	present	-	<1	-	200	0
179	-	present	-	-	-	-	-	1	-	1,200	0
180	-	-	-	-	-	-	present	-	-	300	0
181	-	-	-	-	-	-	-	<1	-	1,000	0
182	-	-	-	-	-	present	present	1	Ga.	18,000	0
183	-	-	-	-	-	present	-	1	-	1,000	40
184	-	-	-	-	-	present	present	<1	-	200	0
185	-	-	-	-	-	-	-	-	-	10	0
186	-	-	-	-	-	present	-	-	-	80	0
187	-	-	-	-	-	-	-	-	-	40	0
188	-	-	-	-	-	-	-	-	-	130	50
189	-	-	-	-	-	-	present	-	-	600	0
190	-	-	-	-	-	-	-	5	-	80	0
191	-	-	-	-	-	-	-	-	-	4,100	0
192	-	-	-	-	-	-	-	-	-	2,800	7
193	-	-	-	-	-	-	-	-	-	2,000	0
194	-	-	-	-	present	-	-	<1	-	2,400	5
195	-	-	-	-	-	present	-	<1	Ga.	4,000	5
196	-	-	-	-	present	-	present	-	-	450	0
197	-	-	-	-	-	-	-	-	-	100	0
198	-	-	-	-	-	-	present	-	-	140	0

TABLE V (Continued)

Lab. no.	Molds	Yeasts	Algae	Diatoms	Actino-mycetes	Slime formers: non-sporing	Slime formers: sporing	sulfate-reducing bacteria per ml	Iron bacteria	Aerobic viable count/ml	Anaerobic viable count/ml
199	-	-	-	-	-	present	-	-	-	19,000	5
200	-	-	-	-	-	present	-	-	-	130,000	3
201	-	-	-	-	-	-	-	-	-	400	1
202	-	-	-	-	-	present	-	4	-	350,000	20
203	-	-	-	-	-	-	present	-	-	25,000	2
204	-	-	-	-	-	-	-	<1	-	600	5
205	-	-	-	-	-	-	-	-	-	1,000	6
206	-	-	-	-	-	present	-	-	Ga.	100,000	2
207	-	-	-	-	-	-	-	-	-	2,000	0
208	-	-	-	-	-	present	-	7	-	1,600	2
209	present	-	-	-	-	present	-	1	Ga.	6,500	2
210	-	-	-	-	-	-	-	-	-	20	1
211	-	-	-	-	-	-	-	-	-	150,000	0
213	-	-	-	-	-	present	-	-	-	700	1
215	-	-	-	-	-	present	-	5	-	370,000	100
221	-	-	-	-	-	-	-	2	-	60,000	10
222	-	-	-	-	-	present	-	-	-	60,000	8
227	-	-	-	-	-	-	-	100	-	320	32
232	-	-	-	present	-	-	-	<1	-	2,400	5
234	-	-	-	-	-	present	-	1	-	21,000	4,000
237	-	-	-	-	-	-	-	15	-	400	4
238	-	-	-	-	-	present	-	<1	Ga.	3,000	1,500
239	-	-	-	-	-	present	-	1	Ga.	700	0
240	-	-	-	-	-	-	-	-	Ga.	1,200	10
241	-	-	-	-	-	present	-	10	Ga.	5,000	110
242	present	-	-	-	-	present	-	-	Ga.	7,500	50
243	-	-	present	-	-	-	-	-	-	9,200	25

TABLE V (Continued)

Lab. no.	Molds	Yeasts	Algae	Diatoms	Actino-mycetes	Slime formers: non-sporing	Slime formers: sporing	sulfate-reducing bacteria per ml	Iron bacteria	Aerobic viable count/ml	Anaerobic viable count/ml
244	-	-	-	-	-	present	-	-	Ga.	500	0
245	-	-	-	-	-	-	-	4	-	100	0
246	-	-	-	-	-	present	-	1	Ga.	6,200	90
247	-	-	-	-	-	-	-	3	-	0	0
248	-	-	-	-	-	present	-	-	Ga.	500	18
249	-	-	-	-	-	-	-	-	Ga.	200	25
250	-	-	-	-	-	-	-	TNTC**	-	600,000	80,000
251	-	-	-	-	-	present	-	1	-	700	45
253	-	-	-	-	-	present	-	-	-	45,000	6
255	present	-	-	-	-	present	present	1	-	350,000	100,000
260	-	-	-	-	-	present	-	9	-	21,000	2,500
268	-	-	-	-	-	present	-	10	-	50,000	3,500
269	-	-	-	-	-	present	-	10	-	500,000	15,000
270	-	-	-	-	-	present	-	200	-	3,700,000	400
271	-	-	-	-	-	present	-	10	-	7,000	1,700
272	-	-	-	-	-	present	-	5	-	9,000	1,200
279	-	-	-	-	-	-	-	-	-	13,000	8,000
281	present	-	-	-	-	-	-	10	Ga.	700,000	10
292	-	-	-	-	-	-	-	-	-	2,200	10
293	-	-	-	-	-	-	-	2	-	50	10
294	-	-	-	-	-	-	-	-	-	650	0
295	-	-	-	-	-	-	-	1	-	1,400	7
296	-	-	-	-	-	-	-	-	-	0	0
297	-	-	-	-	-	-	-	-	-	27	0
298	-	-	-	-	-	-	-	1	-	20,000	50
299	-	-	-	-	-	-	-	-	-	0	0
300	-	-	-	-	-	-	-	-	-	240	0

TABLE V (Concluded)

Lab. no.	Molds	Yeasts	Algae	Diatoms	Actino-mycetes	Slime formers: non-sporing	Slime formers: sporing	sulfate-reducing bacteria per ml	Iron bacteria	Aerobic viable count/ml	Anaerobic viable count/ml
301	-	-	-	-	-	-	-	-	-	40	0
307	-	-	-	-	-	present	-	-	-	250,000	6,500
308	-	-	-	-	-	-	-	-	-	10	0
309	-	-	-	-	-	-	-	-	-	130,000	1,600
310	-	-	-	-	-	present	-	5	-	2,000	500
311	-	-	-	-	-	present	-	-	-	7,000	10,000
312	-	-	-	-	-	present	-	1	-	350,000	300
322	-	-	-	-	-	-	-	-	Ga.	120	0
326	-	-	-	-	-	-	-	-	Ga.	0	0
332	present	-	-	-	-	present	-	-	-	160,000	2
333	-	-	-	-	-	-	-	-	-	400,000	0
335	-	-	-	-	-	-	-	<1	-	110	3
336	-	-	-	-	-	-	-	20	-	550	1
337	present	-	-	-	-	-	-	-	-	11	1
338	-	-	-	-	-	present	present	-	-	350,000	1
339	-	-	-	-	-	-	-	-	-	150	1
340	-	-	-	-	-	-	-	-	-	0	0
341	-	-	-	-	-	present	-	-	-	35,000	2
342	present	-	-	-	-	-	-	-	Ga.	1,300	1
348	-	-	-	-	-	-	-	10	-	2,500	20
349	-	-	-	-	-	-	-	30	-	2,500	10

* Cr. = Crenothrix
Ga. = Gallionella
Si. = Siderocapsa
Sph. = Sphaerotilus

** Too numerous to count.

TABLE VI

Microbiological Analysis of Produced Water.

Lab. no.	Molds	Yeasts	Algae	Diatoms	Actino-mycetes	Slime formers		sulfate-reducing bacteria per ml	Iron bacteria	Aerobic viable count/ml	Anaerobic viable count/ml
						non-sporing	sporing				
68	-	-	-	-	-	-	-	150,000	-	-	-
98	-	-	-	-	-	-	-	1	-	200	4
118	-	-	-	-	-	-	-	<1	-	3,100,000	4,400
119	-	-	-	-	-	-	-	-	-	-	-
139	-	-	-	-	-	-	-	200,000	-	9,600,000	103,000
164	-	-	-	-	-	-	present	200,000	-	2,700,000	1,000
223	-	-	-	-	-	-	-	-	-	-	-

TABLE VII

Microorganisms* Present in Injection Water from Various Sources.

Source of sample	No. of samples tested	Number of samples showing: Molds	Yeasts	Algae	Diatoms	Actino-mycetes
Wells	32	5	3	0	1	0
Lakes and rivers	15	1	0	1	4	0
Storage tanks	14	2	0	0	0	0
Pipes, pumps, etc.	38	10	0	0	3	0
Injection well head	181	26	1	1	1	3
Produced water	7	0	0	0	0	0

* Other than true bacteria

TABLE VIII

Incidence of Slime-Forming, Sulfate-Reducing, and Iron Bacteria in Injection Water from Various Sources.

Source of sample	No. of samples tested	Number of samples showing: Slime formers non-sporing		Slime formers sporing		Sulfate-reducing bacteria		Iron bacteria	
		No.	%	No.	%	No.	%	No.	%
Wells	32	8	25	0	0	15	47	3	9
Lakes and rivers	15	10	67	0	0	6	40	0	0
Storage tanks	14	3	21	2	14	11	79	2	14
Pipes, pumps, etc.	38	7	18	1	3	14	37	3	8
Injection well head	181	51	28	10	6	70	39	26	14
Produced water	7	0	0	1	14	5	72	0	0

TABLE IX

Incidence of Aerobic and Anaerobic Bacteria in

Injection Water from Various Sources.

Source of sample	No. of samples tested	Number of samples showing: Aerobes per ml 10,000 and over		Anaerobes per ml 100 and over	
		No.	%	No.	%
Wells	32	13	41	8	25
Lakes and rivers	15	4	27	4	27
Storage tanks	14	5	36	5	36
Pipes, pumps, etc.	38	20	53	12	32
Injection well head	181	52	29	24	13
Produced water	7	3	43	3	43

TABLE X

Groups of Microorganisms Present in Injection Water from Various Sources.

Source of sample	No. of samples tested	Number of samples showing: Molds, Yeasts, Algae, Diatoms, and/or Actinomycetes		Slime formers, Sulfate-reducers, and/or Iron bacteria		Aerobic viable count >10,000/ml and/or Anaerobic viable count >100/ml		All organisms concerned	
		No.	%	No.	%	No.	%	No.	%
Wells	32	7	22	22	69	16	50	28	88
Lakes and rivers	15	4	27	11	73	6	40	12	80
Storage tanks	14	2	14	12	86	7	50	12	86
Pipes, pumps, etc.	38	11	29	19	50	21	55	28	74
Injection well head	181	29	16	109	60	60	33	132	73
Produced water	7	0	0	5	71	3	43	5	71

TABLE XI

Bacteria Found in Water from Wells of Various Depths.

Lab. no.	Depth in ft.	Slime formers non-sporing	Slime formers sporing	sulfate-reducing bacteria per ml	Iron bacteria	Aerobic viable count/ml	Anaerobic viable count/ml
235	17-33	-	-	-	-	150,000	5
275	27	-	-	-	Ga.*	3,000	1
154	220-352	-	-	1,000	-	83,000	25
317	237	-	-	60	-	300,000	20
225	240-415	present	-	10	-	500,000	16,000
315	242	-	-	< 1	-	4,500	7
314	245	-	-	60	-	15,000	10
316	245	-	-	100	-	80,000	400
82	250	present	-	-	-	112,000	78
153	496	-	-	210	-	2,200	670

* Cr. = Crenothrix
Ga. = Gallionella
Si. = Siderocapsa
Sph. = Sphaerotilus

TABLE XII

Temperature and Bacteriology of Well Water Samples.

Lab. no.	Temperature	Slime formers non-sporing	Slime formers sporing	sulfate-reducing bacteria per ml	Iron bacteria	Aerobic viable count/ml	Anaerobic viable count/ml
154	40°F	-	-	1,000	-	83,000	25
330	41	-	-	-	-	4,000	0
121	44	-	-	2	-	7,000	25,000
275	44	-	-	-	Ga.*	3,000	1
220	45	-	-	20	-	5,000	100
302	46	-	-	-	Ga.	450,000	8,000
304	45	-	-	-	Ga.	350,000	800
282	50	-	-	-	-	200	0
283	50	-	-	-	-	300	0
285	50	present	-	2	-	330,000	8
153	54	-	-	210	-	2,200	670

* Cr. = Crenothrix
Ga. = Gallionella
Si. = Siderocapsa
Sph. = Sphaerotilus

TABLE XIII

Temperature and Bacteriology of Lake and River Water Samples.

Lab. no.	Temperature	Slime formers		sulfate-reducing bacteria per ml	Iron bacteria	Aerobic viable count/ml	Anaerobic viable count/ml
		non-sporing	sporing				
305	32°F	-	-	-	-	5,000	5,000
306	32	present	-	-	-	1,700	100
334	38	present	-	2	-	36,000	200
43	45	-	-	-	-	113	0
44	45	-	-	-	-	110	0
76	54	present	-	-	-	260	16
77	54	-	-	-	-	230	10
74	61	present	-	2	-	750	30
75	61	present	-	-	-	1,000	35
39	71	-	-	30	-	1,800	72

TABLE XIV

Bacterial Populations of Injection Water Samples - Source Wells.

(32 samples tested)

	Number of samples showing:		
Number of bacteria per ml	Desulfovibrios	Aerobes	Anaerobes
0	17	0	10
<1 - 1	1	0	3
2 - 10	6	0	7
11 - 100	6	1	5
101 - 1,000	2	7	3
1,001 - 10,000	0	12	1
10,001 - 100,000	0	4	3
100,001 - 1,000,000	0	7	0
over	0	1	0

TABLE XV

Bacterial Populations of Injection Water Samples - Lakes and Rivers.

(15 samples tested)

	Number of samples showing:		
Number of bacteria per ml	Desulfovibrios	Aerobes	Anaerobes
0	9	0	4
<1 - 1	2	0	0
2 - 10	3	0	1
11 - 100	1	0	7
101 - 1,000	0	7	2
1,001 - 10,000	0	4	1
10,001 - 100,000	0	2	0
100,001 - 1,000,000	0	1	0
over	0	1	0

TABLE XVI

Bacterial Populations of Injection Water Samples - Storage Tanks.

(14 samples tested)

	Number of samples showing:		
Number of bacteria per ml	Desulfovibrios	Aerobes	Anaerobes
0	3	2	6
<1 - 1	0	0	0
2 - 10	4	0	1
11 - 100	2	2	2
101 - 1,000	1	3	4
1,001 - 10,000	0	2	1
10,001 - 100,000	2	2	0
100,001 - 1,000,000	0	2	0
over	2	1	0

TABLE XVII

Bacterial Populations of Injection Water Samples - Ducts.

(38 samples tested)

	Number of samples showing:		
Number of bacteria per ml	Desulfovibrios	Aerobes	Anaerobes
0	24	2	11
<1 - 1	5	0	0
2 - 10	7	1	9
11 - 100	2	1	7
101 - 1,000	0	6	6
1,001 - 10,000	0	8	5
10,001 - 100,000	0	14	0
100,001 - 1,000,000	0	6	0
over	0	0	0

TABLE XVIII

Bacterial Populations of Injection Water Samples - Injection Well Head.

(181 samples tested)

	Number of samples showing:		
Number of bacteria per ml	Desulfovibrios	Aerobes	Anaerobes
0	111	13	84
<1 - 1	30	0	12
2 - 10	29	4	38
11 - 100	8	16	25
101 - 1,000	2	55	9
1,001 - 10,000	0	42	10
10,001 - 100,000	0	29	3
100,001 - 1,000,000	0	21	0
over	1	1	0

TABLE XIX

Genus and Frequency of Occurrence of Iron Bacteria in Injection Waters.

(287 samples tested)

Genera	Number of Samples Positive	Percentage of Samples Positive
Gallionella	27	9.40
Siderocapsa	1	0.35
Sphaerotilus	2	0.70
Sphaerotilus & Gallionella	4	1.40

TABLE XX

Counts for Sulfate-Reducers, Aerobes, and Anaerobes Obtained After Various Incubation Periods.

Lab. No.	Sulfate-Reducers		Aerobes		Anaerobes	
	7 day	21 day	3 day	7 day	3 day	7 day
1	0	0.3	-	-	-	-
2	0.4	0.4	-	-	-	-
3	0.3	0.3	-	-	-	-
4	0.2	0.2	-	-	-	-
10	0	10	-	-	-	-
118	0	0.1	2,900,000	3,100,000	-	-
120	-	-	4,000	20,000	-	-
121	-	-	5,000	7,000	-	-
122	-	-	94,000	106,000	-	-
123	-	-	75,000	107,000	-	-
124	-	-	390	390	-	-
126	0.5	1.0	-	-	-	-
127	0.1	0.1	1,600,000	1,600,000	-	-
130	-	-	3,800	3,800	19	19
131	-	0.2	16,000	21,000	16,000	18,500
133	-	-	550	1,800	-	-
134	-	-	40	220	-	16
135	-	-	160	955	-	-
136	-	-	350	1,500	-	-
138	-	-	11	11	-	-
139	200,000	200,000	8,000,000	9,600,000	-	-
141	3.3	23	50,000	56,500	-	-
142	-	-	-	-	3	3
146	-	-	4,100	10,000	-	-
148	-	-	6,300	37,000	-	-
149	-	-	200	1,000	-	-
150	-	-	300	300	-	-
152	0.5	5	1,000	1,000	-	-
153	180	210	1,800	2,200	-	-
154	20	1,000	76,000	83,000	-	-
157	-	-	420,000	2,900,000	-	-
158	-	-	15,000	15,000	-	-
159	0	1.0	450	800	-	-
160	0	70	1,700	1,700	-	-
161	0	0.3	1,200	1,200	-	-
162	-	-	250	300	-	-
164	2,000	200,000	2,500,000	2,700,000	-	-
165	260,000	TNTC*	2,000,000	2,500,000	-	-
168	0	5	-	-	-	-
169	3,000	20,000	1,000,000	1,000,000	-	-
170	-	-	350	800	-	-
171	-	-	30	30	-	-

TABLE XX (Continued)

Lab. No.	Sulfate-Reducers		Aerobes		Anaerobes	
	7 day	21 day	3 day	7 day	3 day	7 day
172	-	-	200	400	-	-
173	-	-	250	500	-	-
174	-	-	800	800	-	-
175	-	-	300	300	-	-
176			250	250	-	-
177	-	-	20	20	-	-
178	0	0.2	200	200	-	-
179	0	0.5	1,200	1,200	-	-
180	0	0.7	260	300	-	-
181	0	0.3	1,000	1,000	-	-
182	0.1	0.1	16,000	18,000	-	-
183	0	0.7	1,000	1,000	-	-
184	0	0.4	200	200	-	-
185	-	-	10	10	-	-
186	-	-	80	80	-	-
187	-	-	40	40	-	-
188	-	-	130	130	-	-
189	-	-	400	600	-	-
190	0	5	60	80	-	-
191	-	-	2,500	4,100	-	-
192	-	-	2,100	2,800	-	-
193	-	-	1,600	2,000	-	-
194	0	0.1	800	2,400	-	-
195	0	0.1	3,000	4,000	-	-
196	-	-	450	450	-	-
197	-	-	100	100	-	-
198	-	-	100	140	-	-
199	-	-	16,000	19,000	-	-
200	-	-	100,000	130,000	-	-
201	-	-	800	4,000	-	-
202	0	4	350,000	350,000	-	-
203	-	-	25,000	25,000	-	-
204	0	0.1	500	600	-	-
205	-	-	600	1,000	-	-
206	-	-	75,000	100,000	-	-
207	-	-	300	2,000	-	-
208	0	7	500	1,600	-	-
209	0	1	3,000	6,500	-	-
210	-	-	20	20	-	-
211	-	-	150,000	150,000	-	-
212	-	-	400	1,000	-	-
213	-	-	400	700	-	-
215	0	5	300,000	370,000	-	-
216	0	2	50,000	100,000	-	-
217	0	2	20,000	45,000	-	-
218	0	0.5	35,000	40,000	-	-

TABLE XX (Continued)

Lab. No.	Sulfate-Reducers		Aerobes		Anaerobes	
	7 day	21 day	3 day	7 day	3 day	7 day
219	0	2	45,000	90,000	-	-
220	7	20	3,000	5,000	-	-
221	0.1	2	30,000	60,000	-	-
222	-	-	25,000	60,000	-	-
225	10	10	500,000	500,000	-	-
226	2	10	-	-	-	-
227	0	100	210	320	16	32
230	-	-	40,000	40,000	-	-
231	-	-	20,000	20,000	-	-
232	0	0.1	2,400	2,400	-	-
233	0.3	1	38,000	73,000	-	-
234	0	0.5	12,000	21,000	4,000	4,000
235	-	-	130,000	150,000	2	5
237	0	15	300	400	0	4
238	0	0.2	2,700	3,000	1,000	1,500
239	0	0.9	600	700	-	-
240	-	-	1,200	1,200	2	10
241	0	10	5,000	5,000	110	110
242	-	-	6,000	7,500	24	50
243	-	-	8,600	9,200	25	25
244	-	-	500	500	-	-
245	0	3.5	100	100	-	-
246	0	0.5	5,500	6,200	50	90
247	0	3	-	-	-	-
248	-	-	500	500	18	18
249	-	-	200	200	0	25
250	40,000	TNTC	480,000	600,000	80,000	80,000
251	0	1	500	700	13	45
252	0	0.3	7,500	15,500	28	200
253	-	-	45,000	45,000	1	6
255	0.5	0.5	250,000	350,000	5	100,000
259	-	-	50	50	2	2
260	9	9	2,000	21,000	1,250	2,500
261	-	-	90	460	50	50
268	0	10	45,000	50,000	2,400	3,500
269	0	10	330,000	500,000	15,000	15,000
270	20	200	3,700,000	3,700,000	150	400
271	0	10	7,000	7,000	1,700	1,700
272	0	5	9,000	9,000	1,200	1,200
273	15	15	300,000	300,000	-	-
274	1	6	400,000	400,000	-	-
275	-	-	800	3,000	-	-
276	0	0.2	5,000	42,000	-	-
277	-	-	1,500	4,000	-	-
278	-	-	2,000	16,000	-	-
279	-	-	2,600	130,000	-	-

TABLE XX (Continued)

Lab. No.	Sulfate-Reducers		Aerobes		Anaerobes	
	7 day	21 day	3 day	7 day	3 day	7 day
280	-	-	4,000	12,000	-	-
281	0.1	10	10,000	700,000	-	-
282	-	-	200	200	-	-
283	-	-	300	300	-	-
284	-	-	150	150	-	-
285	0.8	2	330,000	330,000	-	-
286	0	2.5	25	25	-	-
287	5	20	400	800	0	400
288	-	-	300	600	5	5
289	-	-	30,000	30,000	400	800
290	-	-	28,000	32,000	0	10,000
291	-	-	7,000	12,000	0	2,000
292	-	-	2,200	2,200	10	10
293	0	2	25	50	10	10
294	-	-	400	650	-	-
295	0	1	300	1,400	0	7
297	-	-	-	-	-	-
298	0	1	20,000	20,000	20	50
300	-	-	150	240	-	-
301	-	-	30	40	-	-
302	-	-	450,000	450,000	0	8,000
303	0	60	100,000	2,100,000	50	50,000
304	-	-	350,000	350,000	2	800
305	-	-	50	5,000	5	5,000
306	-	-	200	1,700	50	100
307	-	-	200,000	250,000	6,500	6,500
308	-	-	0	10	-	-
309	-	-	90,000	130,000	1,600	1,600
310	0	5	2,000	2,000	500	500
311	-	-	5,000	7,000	10,000	10,000
312	0	0.1	250,000	350,000	-	-
314	0	60	15,000	15,000	5	10
315	0	0.3	1,100	4,500	0	7
316	0	100	32,000	80,000	400	400
317	0	60	150,000	300,000	-	-
319	0	2	13,000	28,000	-	-
320	1	15	30,000	300,000	200	1,000
321	-	-	300,000	500,000	7	15
322	-	-	5	120	-	-
323	0	2	700	1,000	3	6
324	-	-	1	6	-	-
329	5	10	20,000	20,000	0	10
330	-	-	3,300	4,000	-	-
331	-	-	5,000	5,200	0	4
332	-	-	160,000	160,000	0	2
333	-	-	300,000	400,000	-	-

TABLE XX (Concluded)

Lab. No.	Sulfate-Reducers		Aerobes		Anaerobes	
	7 day	21 day	3 day	7 day	3 day	7 day
334	0.6	2	10,000	36,000	-	-
335	0	0.3	110	110	3	3
336	0	20	450	550	1	1
337	-	-	3	11	1	1
338	-	-	250,000	350,000	1	1
339	-	-	150	150	1	1
341	-	-	30,000	35,000	2	2
342	-	-	1,300	1,300	1	1
343	-	-	2,000	2,000	0	20
344	-	-	2,500	3,800	-	-
345	-	-	30,000	50,000	-	-
346	-	-	20	20	-	-
347	-	-	120,000	120,000	-	-
348	2	10	2,000	2,500	-	-
349	4	30	1,000	2,500	-	-
350	-	-	1,000	4,000	-	-

* Too numerous to count.

TABLE XXI

Incidence of the Alteration in Viable Count due to Variation in Incubation Time.

Types of Alteration in Viable Count	Sulfate-Reducers (7 to 21 days)		Aerobes (3 to 7 days)		Anaerobes (3 to 7 days)	
	No. of samples tested	No. of samples showing positive	No. of samples tested	No. of samples showing positive	No. of samples tested	No. of samples showing positive
Negative to Positive	95	58 (61%)	186	1 (0.5%)	65	14 (22%)
Insignificant to Significant			186	9 (5%)	65	10 (15%)
No Change	95	10 (11%)	186	69 (37%)	65	27 (42%)

DISCUSSION AND CONCLUSIONS

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The method of microbiological examination used in this investigation was based on the suggestions published by Anderson (1951), Sloat, Clayton, and Ellenberger (1957), and on the recommendation given by the American Petroleum Institute (1958) as to types of organisms which could be damaging in flooding operations. This has been formulated into a more exact qualitative and quantative form by Myers (1958b). Accordingly it is considered that water containing any of the following: an aerobic viable count greater than 10,000 per ml; an anaerobic viable count greater than 100 per ml; any number of slime-forming bacteria, sulfate-reducing bacteria, or iron-depositing bacteria; molds, yeasts, algae, or diatoms, should not be used for injection without treatment.

1. Discussion of methods used and validity of results.

a.) The aerobic and anaerobic viable counts were obtained using pour plates. It is realized that not all the bacteria present in the water samples grew. This may have been due to the lack of required nutrients or to the change in environment. In addition the viable count does not measure the total amount of bacterial protoplasm present since there is always a number of bacteria which are dead. These factors were taken into consideration when the above mentioned limits for the microbiological quality of injection water were devised. If the water has been treated with bactericides but not subsequently treated by a proper filtration or sedimentation

process an erroneous estimate of the amount of bacterial protoplasm being introduced into the formation will result if the viable bacterial count is the sole measure of suitability. The determination of the viable count of anaerobic bacteria is particularly important in that there is a good chance of growth and multiplication of anaerobes in injection waters where oxygen has been removed but other nutrients are present. The pipeline between the treating plant and the injection well head and stagnant places in an injection well are most vulnerable to the activities of anaerobic bacteria. In addition there is also the possibility that the anaerobes may grow and multiply in the formation itself.

The viable bacterial count was recorded after three and seven days of incubation to establish the best incubation period (see Tables XX and XXI). It was found that in the majority of cases there was no significant increase in counts from three to seven days, but in twenty percent of the tests there was a significant increase. In the cases where an increase in count was recorded it was due to the fact that in the first three days of incubation some bacteria produce only tiny colonies which are easily overlooked. However, it is not advisable to postpone all counting until after seven days of incubation, because in many cases the individual colonies grow too large to yield reliable counts on plates having fifty colonies or more. In addition one rapidly spreading colony can overgrow the entire surface of the medium in this time. Thus all viable bacteria counts in

this study were based on counts obtained after three as well as seven days of incubation. Examination for the presence of molds, yeasts, and actinomycetes was done on the third and seventh day of incubation for similar reasons.

b.) The detection of slime formers was based on the assumption that bacteria producing a large amount of slime while in water will continue to do so on the surface of freshly poured medium. This is highly presumptive and it is very likely that in a majority of cases the production of slime will cease when the environment has been changed. Another possibility exists that organisms producing slime on nutrient agar medium will not do so in the water from which they were isolated. A more exact method of detecting bacteria that produce slime in injection water is to prepare a direct smear of the water to be tested and observe whether the organisms show the presence of capsules. This method, however, would be impractical for our investigation since very high concentrations of bacterial cells are required in the sample in order that any cells will be seen in a microscopic field.

c.) The sulfate-reducing bacteria are sensitive to changes in salinity, hydrostatic pressure, and redox potential. In our study all the injection waters had a low salt content and thus there is very little difference between the salinity of the injection water and that of the medium. As far as pressure is concerned it would become significant in the case of waters obtained from deep wells. But the wells from which our

samples were taken are not sufficiently deep to have high hydrostatic pressure. The principal factor which many workers have had to cope with in order to obtain growth of sulfate-reducing bacteria has been proper redox potential. This problem was solved by Allred, Mills, and Fisher (1954) who prepared a medium using a mixture of ascorbic acid and sodium thioglycollate. This is the medium which was modified in our department and employed in this investigation.

The time of examination of the SR-medium inoculated with injection water was set at seven and twenty-one days of incubation. It was noticed that in 58 out of 95 cases (or 61%) growth appeared after seven and before twenty-one days of incubation (see Table XXI). In no instance where growth did not appear before twenty-one days of incubation did it appear after. For this reason an early microbiological report can be given after seven days of incubation but a final report should not be given until twenty-one days have elapsed.

Since it was observed that certain organisms (Salmonella paratyphi A) produce yellow colonies in the SR-medium in fourteen days and that these colonies turn black before twenty-one days of incubation it would seem advisable to examine the medium at fourteen days of incubation as well as seven and twenty-one to avoid mistaking these colonies for Desulfovibrio.

d.) Because there is no single simple medium for growing iron bacteria the detection of these organisms is based on the preparation and examination of microscopic slides (see "Staining technique", page 25). In this procedure the "microscopic

factor" has to be considered. This factor is based on the fact that when a smear is prepared only a certain portion of the material used for making the smear can be seen at any given instant. Thus unless the concentration of bacteria in the original material is relatively high and unless the cells are evenly distributed the examination of a single microscopic field or even several fields may give a negative result. It is recognized that relatively large numbers of bacteria may be overlooked using this procedure. However, in the absence of more precise methods for detecting these organisms it was attempted to overcome to some extent the weakness in the procedure by concentrating the bacteria from 50 ml of sample into 1 ml by centrifugation. As far as stalks or filaments of iron bacteria are concerned they could be detected on a smear at a lower per ml concentration than single bacterial cells, such as Siderocapsa.

Recognition of the weakness inherent in the microscopic method of detecting iron bacteria instigated attempts to prepare a culture medium which would enable one to detect this organism at lower per ml concentration than is possible with the microscopic technique. Attempts to obtain artificial cultures were undertaken using Wolfe's medium (1958), Lieske's medium (1911), and a modification of Lieske's medium as described by van Iterson (1958). The inoculum was obtained from injection waters showing presence of Gallionella stalks, spring water from White Mud Creek (Edmonton) and well water from Wabamun Lake area where the

organisms were detected; in addition a culture kindly sent by Dr. R. S. Wolfe of the University of Illinois was used. These experiments were unsuccessful and it is suggested that this problem might well be the subject of future research.

The results of the microscopic examinations show that out of thirty-four cases in which iron bacteria were detected thirty-one were Gallionella, six were Sphaerotilus, and in one case Siderocapsa was observed (see Table XIX). The reason that Siderocapsa was detected only in one case could be because of the microscopic factor which is involved in the microscopic examination. Yet, the ease of detecting Gallionella stalks as well as Sphaerotilus filaments can be regarded as equal which indicates that Gallionella species are more frequent in occurrence and thus more likely to cause trouble.

2. Discussion of results obtained and general conclusions.

With regard to the conclusions which can be validly drawn from these results one should exercise extreme caution. Sampling at the injection well head is done more or less routinely, yet it is suspected that the majority of operators submit samples for microbiological analysis only when a pressure build up is noticed and therefore the samples analysed in this investigation probably are not truly representative of the quality of injection water being used.

The results in Table X indicate that there is little difference between surface water and well water as to suitability for injection purposes. From a bacteriological point of view it was expected that surface water would be less suitable for flooding operations than well water. Our results are contrary to this expectation. These waters do not differ greatly from one another even when taking into consideration the types and numbers of organisms occurring (see Table X). It is suspected that this could be due to the fact that some samples obtained from lakes and rivers were taken during winter months. One should also bear in mind that a relatively small number of samples were tested.

Slime-forming bacteria were present in water samples obtained from lakes and rivers almost three times as frequently as in samples taken from water wells (see Table VIII). In neither water source were spore-producing slime formers found although these organisms were detected in the rest of the injection system. The frequency of occurrence of slime

formers in samples obtained from storage tanks, ducts, and injection well head is not very high. In our opinion, however, their plugging property is too effective to tolerate their presence at the injection well head.

Sulfate-reducing bacteria have been detected in both water sources (well water and surface water) at approximately the same frequency (see Table VIII). Water samples obtained from storage tanks almost invariably showed the presence of Desulfovibrio (79% positive). This is a very undesirable situation because these organisms cause corrosion of metal equipment as well as plugging of the formation sand-face with bacterial protoplasm and corrosion products. Samples obtained from storage tanks show a higher frequency of Desulfovibrio present than in samples obtained from injection well heads (see Table VIII). This situation is not due to the effective treatment of the water between the storage tanks and the well heads but to the fact that in many systems, where storage of injection water is not employed, the sulfate reducers are absent.

Iron bacteria were never observed in smears prepared from lake or river water, but this does not necessarily mean that they were absent. These bacteria were detected in well water and water samples obtained from storage tanks, ducts and injection well heads (see Table VIII).

There can be no correlation drawn between the presence of Gallionella and the depth of the water wells from which the samples were taken since these organisms were detected only in three out of thirty-two cases and the depth of the

well was given only in one case (see Table XI). In any system where Gallionella stalks were present the treatment employed never erradicated the organisms completely. The organisms always reappeared after a lapse of time. In one instance filtration was employed in which case a water sample obtained immediately after treatment appeared to be sterile (sample No. 325), but a sample taken from the farthest point of that injection system showed the presence of Gallionella stalks as well as an aerobic viable count of 120 organisms per ml (sample No. 322).

Iron bacteria have been shown experimentally to cause effective plugging (Merkt, 1943). A practical example of such plugging was experienced at Joffre, Alberta. Injection wells became plugged with Gallionella stalks and even after cleaning operations some of these wells were never able to take water at the previous rate.

Table X shows the presence of molds, yeasts, algae, diatoms, and actinomycetes in injection waters. From this table it can be seen that these organisms are found throughout the injection system, but they occur seldom. It is of significance to note that the percentage of positive samples is higher for samples taken from the water source than for samples obtained from storage tanks and injection well heads. Thus, although the method of detecting the above organisms may not be exacting, the results indicate that their presence is not a major factor in causing accumulation of organic matter. This, however, does not mean that we should disregard their presence.

With respect to source-waters containing an insignificant number of molds, yeasts, algae, diatoms, actinomycetes, or a low viable bacterial count, some bactericide or growth inhibitor should be used intermittently in order to avoid the possibility of multiplication. This would be of more importance where water is transported through longer distances or when it has to be stored. Treatment is imperative if either slime formers, sulfate reducers, or iron depositing bacteria are present.

SUMMARY

SUMMARY

1. Eighty-eight percent of water samples coming from source-wells and eighty percent of water samples obtained from lakes and rivers are unsuitable for injection without treatment.
2. The most damaging bacteria in flooding operations (slime formers, sulfate reducers, and iron bacteria) were found in 69% of samples coming from wells and in 73% of samples obtained from lakes and rivers.
3. High viable bacteria counts (aerobes and/or anaerobes) were recorded in 50% of samples taken from wells and 40% of samples received from lakes and rivers.
4. Molds, yeasts, algae, diatoms, and/or actinomycetes were detected in 22% of samples which came from wells and in 27% of samples taken from lakes and rivers.
5. Slime formers were detected three times more frequently in samples taken from lakes and rivers than in samples obtained from wells.
6. Spore-producing slime formers were not found in either of the water sources (surface water or well water) but they were detected elsewhere in the injection system.
7. Sulfate reducers were detected with approximately equal frequency in well water and surface water.
8. Water samples obtained from storage tanks almost invariably showed the presence of sulfate-reducing bacteria.
9. Iron bacteria were detected at various sampling points of different injection systems.

10. The frequency of re-occurrence of iron bacteria in water systems which are occasionally treated indicated the difficulty in erradicating these microorganisms once they have become established.

11. Molds, yeasts, algae, diatoms, and/or actinomycetes occur in injection waters at such a low frequency and in such small numbers that they may be considered insignificant.

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APPENDIX I

MICROBIOLOGICAL REPORT

Laboratory Number ..... Date of Sampling ..... Time .....
Date of Receiving ..... Time .....
Refrigerated in Transit: Yes .... No .... Temp. .....°
Sampling Tap Sterilized: Yes .... No ....

Name of Source .....
Location of Source: LSD ... Sec ... Twp ... Rge ... W ... Mer. KB Elevation ....
Field or Area ..... Pool ..... Grd Elevation ....
Sample obtained from .....
Depth interval sampled ..... feet to ..... feet.
Temperature of sample at time of collection ..... °F or ..... °C.
Name of Zone and Formation sampled .....
Sample obtained by .....(Individual).
For ..... (Organization).

	Immediate Microscopic Examination	Cultural Examination at 20°C		
		3 day	7 day	21 day
FUNGI Penicillium, Molds, e.g. Aspergillus.	.....			
Yeasts, e.g. Torula.....	.....			
ALGAE Flagilaria, e.g. Chlorella.				
DIATOMS e.g. Navicula.				
BACTERIA * (Slime-forming)				
Non-spore-forming, e.g. Pseudomonas.	.....			
Spore-forming, e.g. B.subtilis.	.....			
CORROSIVE (Sulfate-reducing) ..... e.g. Desulfovibrio	.....	.....		
IRON-DEPOSITING Siderocapsa, Crenothrix, e.g. Gallionella.				
ACTINOMYCETACEAE * Nocardia, e.g. Actinomyces.	.....			
UNUSUAL TYPES OBSERVED				
AEROBIC VIABLE BACTERIAL COUNT	.....			
ANAEROBIC VIABLE BACTERIAL COUNT	.....			
TOTAL COUNT				

* Surface inoculation - see procedure.

Bacteriologist .....

Date of Report .....

APPENDIX II

ORGANISMS USED FOR TESTING SPECIFICITY OF IRON STAIN

BACTERIA

Aerobacter aerogenes

Acetobacter aceti

Bacillus circulans

Bacillus subtilis

Corynebacterium diphtheriae type *gravis*

Corynebacterium diphtheriae type *mitis*

Corynebacterium diphtheriae type *intermedius*

Corynebacterium diphtheriae type *xerosis*

Escherichia coli

Klebsiella sp.

Micrococcus sp.

Paracolobactrum sp.

Proteus mirabilis

Proteus morganii

Proteus vulgaris

Pseudomonas aeruginosa

Salmonella abortivoequina

Salmonella choleraesuis

Salmonella oranienburg

Salmonella paratyphi A

Salmonella paratyphi C

Salmonella schottmuelleri

Salmonella typhosa

Sarcina sp.

Serratia marcescens

Shigella flexneri

Staphylococcus aureus

Staphylococcus aureus white variety

Streptococcus lactis

Streptococcus faecalis

Streptococcus mitis

Streptococcus pyogenes

Streptococcus salivarius

FUNGI

Candida albicans

Gelasinospora tetrasperma

Neurospora tetrasperma

Penicillium chrysogenum

Penicillium columnare

Penicillium flavidorsum

Saccharomyces cerevisiae

Streptomyces flaveolus

Streptomyces lavendulae

plus seven cultures isolated from injection waters.

APPENDIX III

ORGANISMS USED FOR TESTING SPECIFICITY OF SR-MEDIUM.

BACTERIA

Aerobacter aerogenes

Acetobacter aceti

Alcaligenes faecalis

Bacillus circulans

Bacillus subtilis

Clostridium histolyticum

Clostridium perfringens

Clostridium sporogenes

Corynebacterium diphtheriae type gravis

Corynebacterium diphtheriae type intermedius

Corynebacterium diphtheriae type mitis

Escherichia coli

Micrococcus sp.

Paracolobactrum sp.

Proteus mirabilis

Proteus morganii

Proteus vulgaris

Pseudomonas aeruginosa

Salmonella abortivoequina

Salmonella choleraesuis

Salmonella oranienburg

Salmonella paratyphi A

Salmonella paratyphi C

Salmonella schottmuelleri

Salmonella typhosa

Sarcina sp.

Serratia marcescens

Shigella flexneri

Staphylococcus aureus

Staphylococcus aureus white variety

Streptococcus faecalis

Streptococcus lactis

Streptococcus pyogenes

FUNGI

Candida albicans

Saccharomyces cerevisiae

Streptomyces flaveolus

plus seven cultures isolated from injection waters.